On the morphology and organization of the eye of unfed adult
*Rhipicephalus evertsi mimeticus* (Acari: Ixodidae)

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Accepted December 15, 1989

Abstract. Investigations by scanning and transmission electron microscopy and freeze-etching revealed that eyes of adult *Rhipicephalus evertsi mimeticus* consist of a lens and photoreceptor cells, which are separated by an acellular layer and the hypodermis. The lens contains numerous pore channels, which open beneath the epidermis of the outer portion, converge uninterrupted to the inner closure and end in approximately 420 pore fields. The inner closure of the lens is formed as a deep circular invagination. Beneath the hypodermis and perpendicular to the lens, a group of approximately 20 highly differentiated photoreceptor cells in a rosette-like arrangement is localized within the invagination of the lens. Each photoreceptor cell is characterized terminally by numerous, tightly packed and parallel-running microvilli, which are oriented perpendicularly to the lenticular pore channels.

A fundamental prerequisite for physiological studies of vision is knowledge of the morphology and organization of the optical radiance and/or light-receiving exteroceptors. The paired, integumental surface structures, referred to as eyes, which are situated at the lateral border of the scutum and the transition area to the alloscutum have been interpreted to be peripheral organs of vision. They are present in the ixodid genera *Amblyomma*, *Anomalohimalaya*, *Boophilus*, *Cosmiomma*, *Dermacentor*, *Hyalomma*, *Margaropus*, *Nosomma*, *Rhipicentor* and *Rhipicephalus*.

The attribution of these structures as light-perceiving organs is based on histological and ultrastructural investigations; however, these were carried out only on the eyes of *A. americanum* (Phillis and Cromroy 1977), *H. asiaticum* (Ivanov and Leonovich 1983; Leonovich 1979), and *H. dromedarii* (El Shoura 1988), all of which were consistently interpreted to be dioptric systems consisting of a lens and photoreceptive neurons. Previous histological studies or morphological descriptions and functional interpretations of eyes of ixodid tick genera (Bonnet 1907; Dethier 1957; Douglas 1943; Gossel 1935; Nosek and Campor 1973; Schulze 1948, 1951; Vitzthum 1925) are mainly of historical interest because of inadequate methodology.

Additional morphological studies of eyes of other ixodid genera should therefore be pursued, especially with scanning electron microscopy. In the following study the morphology, especially the fine structure and organization, of the eyes of unfed adult *Rhipicephalus evertsi mimeticus* were investigated. This two-host tick species of veterinary importance (Gothe et al. 1986a–d) is native to the southwestern part of the Ethiopian fauna region and predominantly parasitizes herbivorous domesticated and wild animals. It is unique among the genus *Rhipicephalus* because of its orbited eyes.

Materials and methods

Scanning electron microscopy

For scanning electron microscopy (SEM) the eyes, including orbita and sublenticular tissue, of 203 unfed adult *R. evertsi mimeticus* were dissected, immediately fixed in 70% ethanol and then broken horizontally, tangentially and sagitally with a scalpel blade. Dehydration was achieved with acetone in a 70%, 90%, 95% and 100% series. After critical-point drying (Balzers CPD 020), the samples were placed in a sputter device (Balzers SCD 040), coated with approximately 12 nm gold/palladium and examined in a Zeiss digital scanning electron microscope (DSM 950).

Transmission electron microscopy

Ultra- and semithin sections. Analogously to SEM, the eyes of 98 adult ticks were removed for transmission electron microscopy (TEM), immediately fixed in paraformaldehyde-glutaraldehyde (Karnovsky 1965) in 0.1 M cacodylate buffer (pH 7.4) for 3 h at 4°C. Thereafter, the specimens were rinsed four times in cacodylate buffer, postfixed in 1% buffered osmium tetroxide for 1 h, washed again with buffer and dehydrated in a series of increasing concentrations of ethanol for 15 min each. Intermediate block contrasting...
with 1% uranyl acetate was carried out at the dehydration stage of 70% ethanol at 4°C. After dehydration in 90% ethanol, the samples were twice placed in absolute alcohol and in propyleneoxide for 30 and 15 min, respectively. The samples were embedded in Durcupan ACM, with subsequent polymerisation for 48 h at 60°C.

For light microscopy, either 1- to 2-μm-thick semithin sections were prepared and stained with methylene blue-azure II or the isolated lens was immediately investigated with a phase-contrast microscope (Leitz).

Results

Morphology of the lens

SEM revealed that eyes corresponded in both sexes and appeared as paired, convex, hemispherical structures dorsally situated between the second and third pair of legs on the lateral scutal margin (Fig. 1). They were clearly delineated from the surface level of the scutum by an orbita (Fig. 2). Their greatest diameter was in the deepest area of the orbita and amounted to an average of 131 and 132 μm for male and female ticks, respectively. The maximal height from the base of the orbita was also similar in male and female ticks and reached, on average, 56 and 47 μm, respectively. The surface of the projection that should be considered a part of the lens was uniformly and uninterruptedly smooth and homogeneous. Few irregularly distributed campaniform-like sensilla were embedded at the transition to and in the orbita (Fig. 2).

In light microscopic examination, the lens appeared as a very transparent, almost cork-shaped insertion in the cuticula, traversed by numerous fine striae that converged to the inner closure. It was externally markedly convex and internally slightly concave (Fig. 3). On the level of the greatest diameter, the outer cuticula rose to form a cuff with ridge like strands running from the orbita, which surrounded the outwardly arching part of the lens (Fig. 2). Channels 0.05–0.1 μm in diameter started immediately below the epicuticula of the curved, hemispherical outer portion of the lens. The channels were surrounded by a matrix and converged uninterruptedly to the interior of the lens (Fig. 4). In the middle of the lens their diameter approached 0.2–0.5 μm. They ended in approximately 420 clearly delineated pore fields as bundles of 13–30 channels with an interior diameter of 0.4–0.5 μm (Fig. 5, insert). The inner closure of the lens was concave, formed as a 34-μm-deep, circular invagination; however, the average diameter of 92 μm was considerably smaller than the widest external diameter (Fig. 5).

Peripherally the lens formed a 57-μm-wide and 31-μm-high ridge. Pore fields were confined to the invagination of the inner lens up to the border of the ridge. Centrally in the invagination, the pore fields measured an average of 2.6 μm wide and 3.2 μm long, with 13–18 channels/pore field. With increasing distance from the center, pore fields gained continuously in the size and number of channels and reached maximal values of 9.3 μm in length and 5 μm in width, with 20–28 channels/pore field at the level of the ridge (Fig. 5, insert). The total number of channels/lens calculated on the basis of the total number of pore openings was almost equal in both sexes. On average, 8113 and 7871 channels were present in male and female ticks, respectively.

According to ultrathin sections and freeze-etch replicas, the lens consisted of numerous pore channels embedded in a very fine granular, homogeneous ground substance (matrix). The outer boundary of the channels was not formed by a unit membrane. Freeze-etch replicas proved that the channels were tunnel-shaped, running convergently to the inner closure of the lens.

Morphology of the sublenticular tissue

Immediately below the invagination of the inner lens, an acellular (approximately 4 μm thick), wavy, plaque-like or laminar layer was present (Fig. 6), directly followed by the one-cell-layer-thick hypodermis, which exhibited numerous spongy cytoplasmic projections in the vicinity of the acellular layer and rested on a basement membrane. Inwardly these projections became looser and formed vesicles (Fig. 6). Filaments from the sublenticular tissue communicated with the inner openings of the pore channels, extending deep into them and almost completely filling their lumena (Fig. 6).

Beneath the basement membrane of the hypodermis and perpendicular to the lens, a group of approximately 20 highly differentiated cells in a rosette-like arrangement were localized within the concave invagination of the inner closure of the lens (Fig. 7, insert). The cells were wedge-shaped, up to 17 μm long and maximally 10.5 μm wide and were ultrastructurally identical, with no difference in arrangement seen between neighbouring cells. Every photoreceptor was terminally characterized by numerous, tightly packed and parallel-running microvilli up to 200 nm in diameter (Fig. 9), which were oriented perpendicularly to the lenticular channels. They consistently bordered the microvilli region of other cells at different angles although separated from them by a double membrane originating from the mesaxon. The base of the microvilli was conspicuous because of its deep invaginations as well as aggregations of glycogen-like inclusions (Fig. 7). Numerous crista-type mitochondria, intracellular channels, endoplasmic reticulum and membrane-bound vesicles were distributed in the rest of the photoreceptor cell soma. The nucleus, always with a distinct nucleolus, was situated peripherally. A double membrane surrounded each neuron and a neural lamella, all photoreceptor cells. In addition, each photoreceptor cell was separated by septa, which were connected with the membranes surrounding the axons and condensed towards the centre of the rosette, releasing fibrils into the microvilli region (Fig. 8).

Discussion

Comparing our results on the morphology and organization of eyes of male and female *R. evertsi mimeticus* with analogous investigations in *A. americanum* (Phillis